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A RHIZO-FILTRATION-BASED APPROACH WITH SELENIUM FOR BIOFORTIFICATION OF OIL CANOLA (*Brassica napus*)

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ABSTRACT

A pots experiment was carried out at the experimental Farm of Kafer El-Hamam Agric. Res. Station El Sharkia Governorate, Egypt in season 2019 to evaluate using rhizo-filtration for biofortification of oil canola (*Brassica napus* L.) cv. Serw 4 with selenium. Canola plants which planted in water culture were treated with selenium (as sodium selenate “Na_2SeO_4” form) at a concentration of 0 (control), 1, 1.5 and 2 mgL^{-1}. The results showed that there were statistically significant differences between the treatments on the studied attributes with superiority canola plants treated with 2 mg Se L^{-1} to other treatments. The relative increase in total dry weight of canola were 11.36, 21.99, 31.04% of canola plants treated with 1, 1.5 and 2 mg Se L^{-1}, respectively comparing with control. The seed oil content percent were 37, 40, 43 and 48% of canola plants treated with 0, 1, 1.5 and 2 mg Se L^{-1}, respectively. Total chlorophyll of canola was 1.94, 2.12, 2.35 and 2.63 mg g^{-1} fresh weight of canola plants treated with 0, 1, 1.5 and 2 mg Se L^{-1}, respectively, while the proline was 4.45, 6.55, 6.95 and 7.35 μmol g^{-1} fresh weight of canola plants treated with 0, 1, 1.5 and 2 mg Se L^{-1}, respectively. Total Se uptake by plants were 7.54, 206.87, 315.92 and 440.8 μg pot^{-1}, while Se content in oil were 0.57, 15, 26.95 and 41.89 μg pot^{-1} of canola plants treated with 0, 1, 1.5 and 2 mg Se L^{-1}, respectively.

1-INTRODUCTION

Selenium (Se) is one of the major deficient micronutrients and various reports indicated that more than 15% of the world population is selenium deficient **(Grusak and Chakmak, 2005; Thacker et al., 2006).** It is an important for many plants element, **(Lyons et al., 2009; Gupta and Gupta 2017).** The difference between Se insufficiency and harm is slender for human being and animals, **(Fordyce et al., 2000).** Loss of Se will have a greater impact on human health. Such loss is expected to increase global Se deficiency in humans. The sources for humans are the plants and livestock.

(Jones et al., 2017). The amount of selenium in diet is diverse and depends on the location in which plants were growing and animals were living **(Schiavon et al. 2020)**.The Recommended intakes of selenium for adults vary; WHO recommend 30to40 μg/day^{-1} **(WHO, 2004)** and **(Thomson, 2004).** Recommended 55 μg/day^{-1} for USA and Canada

Low serum Se levels in humans have been associated with negative consequences (**Arthur et al. 2003;Hoffmann and Berry 2008)** and in extreme cases, diseases related to Se-deficiency **(Fairweather-Tait et al. 2011)**. Se supplementation may alleviate these health concerns **(Steinnes 2009).**Selenium is a necessary supplement for humans and animals **(Kaur et al., 2014).** Selenium containing proteins play a role in proliferation DNA combination, and contamination **(Hatfield et al., 2014).**

Selenium consisting plants might be used in food and can be utilized to mitigate selenium insufficiency **(Banuelos and Dhillon, 2011a).** To combat the deficiency of selenium biofortification can be performed by suppling plants with Se (**Banuelos and Lin, 2009)**. Plants of phytoremediation might be utilized as manure in Se biofortification **(Banuelos et al., 2015).**

Rhizo-filtration is phytoremediation using hyper accumulator plants to absorb heavy metals from soil. Three techniques of Se-biofortification were: hydroponic culture, soil fertilization, and foliar spray. The highest reported Se concentration in the Brassicaceae ranged from1,200 to 1,800 μg Se g^{-1} DW in broccoli. **(Banuelos, et al 1997; Verma et al. 2006, Lee and Yang 2010., and Abdel-Salam et al.,2015)**.Canola (*Brassica napus L.)* is one of the most important edible oil crops.It is grown in more than 120 countries around the world. **(Przybylski et al., 2005).** Rapeseed is important crop and is the third source of oil after soybean and palm oil **(El-Beltagi & Mohamed, 2010).** Canola is a secondary accumulator of selenium with Se of several hundred mg Se/kg DW. The effects that selenium may have on canola, and possibly other crops, are relevant to farmers who may be growing plants in selenium-rich soil .The impacts of dietary selenium, the role of selenium in plant growth, and the use of plants for phytoremediation of selenium-rich soil are important Selenium metabolism in higher plants and the use of crop plants for phytoremediation and as a source of dietary selenium have increased dramatically over the past 10 years **(Bañuelos et al. 1990, 1992, 1993, 1997a, 1997b, 1998; Terry et al. 2000. Wiesner-Reinhold et al.2017,)**

The current study aims at assessing Se accumulation in canola under hydroponics conditions and the effects that this element has on plant growth.

2-MATERIALS AND METHODS

A pots experiment was carried out at the experimental Farm of Kafer El-Hamam Agric. Res. Station El Sharkia Governorate, Egypt in season 2019 to evaluate using rhizo-filtration for biofortification of oil canola (*Brassica napus* L.) cv. Serw 4 with selenium. Sodium hypochlorite solution (1%) was used to sterilize Seeds were for 15 minutes and washed thoroughly with distilled water before use. Initially canola seeds were grown in trays (sand culture) in a greenhouse illuminated with natural light. Nursery was irrigated with distilled water every day and half strength Hoagland solution was applied every week. After, two-weeks seedlings of uniform size were transplanted An experiment was conducted using setups of hydroponic culture in container pots **(Cooper, 1975 , Fehr and Caviness, 1977 ,Dushenkov et al., 1997 and Dushenkov and Kapulnik, 2000).** Eash pot (50-cm diameter; 40-cm height) was filled with 10 L of half strength Hoagland solution **(Epstein 1971 and Menge et al, 2001)**.**Figure 1** shows a drawing of the set-up. The experimental design was a randomozed complete block in 3 replicates. In each pot , a bottomless PVC cylinder (17-cm diameter ; 50-cm height) was immersed into the pot. The cylinder was in two parts separated by a perforated PVC/PS double layered disc situated within the interface between the hydroponic water and the space above where the transplant is anchored. Plants (2 seedlings pot^{-1}) were placed supported by, 10-cm thick fluffy perlite/vermiculite mixture .Thus plant roots would grow through the perforations to enter and get immersed into the culture solution to take up water and elements from it. An air pump was immersed into the pot to keep the solution constantly aired and provide oxygen for root growth .Therefore the lower part contained the hydroponic solution and the upper accommodated the canola seedlings. plants had Se added to the nutrient as sodium selenate(Na_2SeO_4) four different concentrations as follows: T0 = 0, T1= 1.00, T2=1.50, and T3= 2mg L^{-1}these plants were grown at 2 mg L^{-1} selenium, and is a concentration commonly reported in the literature **(Bañuelos et al. 1993, 1996).** A stock solution of analytical grade cadmium chloride sodium selenate(Na_2SeO_4) (1000 mg/l^{-1}) was prepared in distilled water and was later diluted as required. The volume of the solution was kept constant by adding deionized water to compensate for water lost through evapotranspiration .The pots were kept outdoor under natural environmental conditions **(Abhilash et al., 2009).**

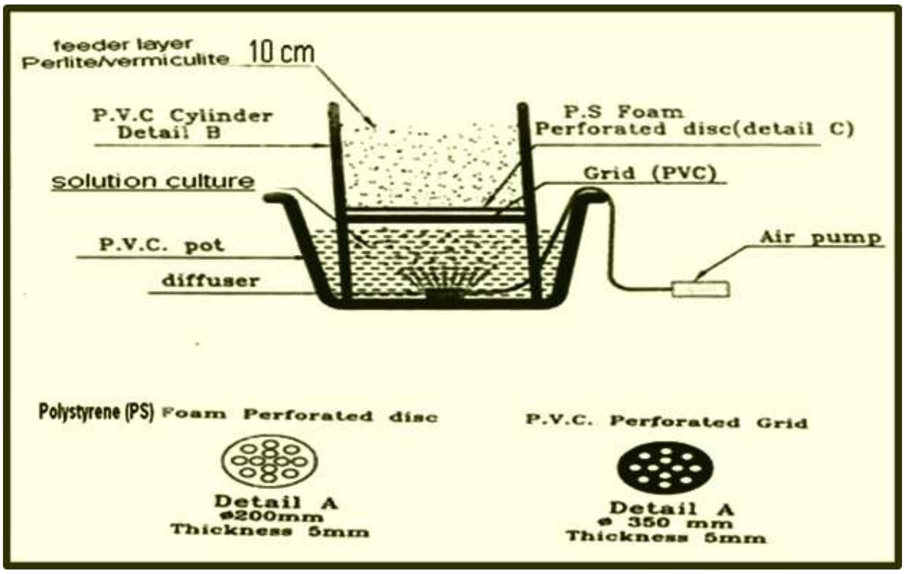

Fig. 1. Schematic representation of rhizo-filtration where Se is uptake from water culture by canola plants

Proline content was determined according to the method adopted by **(Bates et al., 1973).** Total chlorophyll as well as chlorophyll a and b concentrations were calculated according to **Amon (1949).** At the end of the experiment (120 days) , plants were (shoots, roots, and seeds) were dried at 70 °C until constant weight. Seed oil content was determined, by Soxhlet extraction using diethyl ether **(AOAC, 1980).**

2-1Selenium analysis:

Selenium was determined by hydride generation atomic absorption spectrometry (HGAAS).

Seed translocation factor (Seed TF) was calculated according to **Ebrahimi et al. (2015)** Using the following equation

$$\mathbf{Seed\ TF} = \frac{\mathbf{Seed\ Se\ content}}{\mathbf{Shoot\ Se\ content}}$$

3-RESULTS AND DISCUSSION

3-1Plant growth:

Data in **Table 1 and fig2** indicate that increasing Se level caused a gradual increase in plant . Total the dry weight of plant growth was 21.65, 24.11, 26.41 and 28.37 for treatments of 0, 1.00, 1.50, and 2mg L^{-1} Se respectively. these results are in harmony with those recorded by **Singh et al. (1980)** who found that 0.5 mg kg^{-1} Se as selenite stimulated growth and dry-matter yield of Indian mustard (*Brassica juncea* L.). **Hasanuzzaman et al., (2010)** reported that Se, applied at 2.5 mg L^{-1},

enhanced growth and antioxidative capacity of mono- and dicotyledonous plants. The results are in agreement with the findings of **Rı´os et al. (2009) and Ramos et al. (2010)** who showed that the effect of Se on plant growth depends on Se in the growth solution **Hartikainen et al. (2000)** reported that at low contents, Se acts as an antioxidant by diminishing the lipid peroxidation, whereas at high contents it acts as a pro-oxidant by increasing the accumulation of thiobarbituric acid reactive substances

Table 1. Effect of the studied treatments on dry weight of shoots, roots and seeds and oil content of seeds

Treatment	Shoots, g pot^{-1}		Roots, g pot^{-1}		Seeds, g pot^{-1}		Total dry weight, g pot^{-1}	Seed oil content, %
	Shoots	RI*	Roots	RI*	Seeds	RI*		
T0	15.14	-	2.56	-	3.95	-	21.65	37
T1	17.5	15.58	2.74	7.03	4.37	10.63	24.11	40
T2	18.7	23.51	3.14	22.65	4.57	15.69	26.41	43
T3	20.15	33.09	3.45	34.75	4.77	20.75	28.37	48
LSD at 5%	2.54	-	1.15	-	1.76	-	3.16	

***RI= Relative Increase %=[(treatment weight- control weight) / control weight] x %100**

****Seed yield values are presented as air dried weight and seed oil contents are presented on a zero moisture basis**

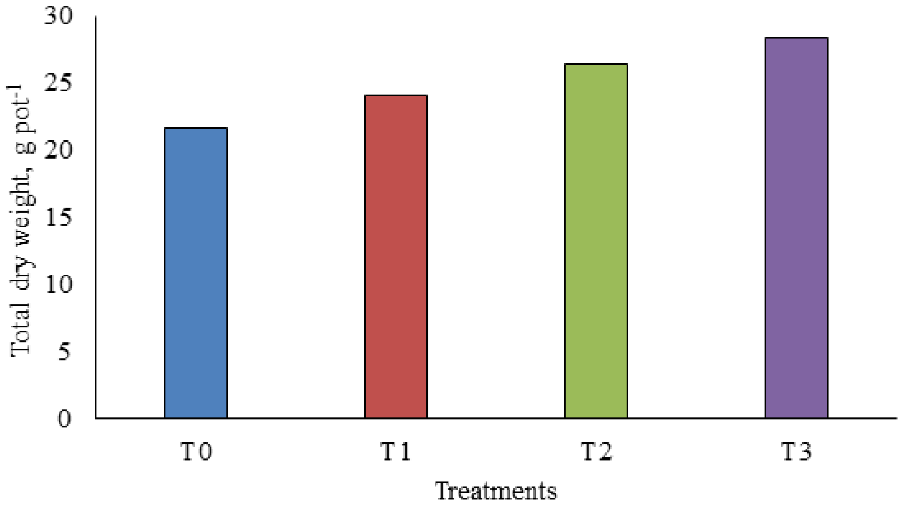

Fig. 2. Effect of the studied treatments on total dry weight

3-2Biochemical constituents of leaves

A addition of selenium in (**Table 2) ,show** that increasing the rates of Se from 1.00 to 2.00 mg/L^{-1} led to significant increase in proline content from 6.55 to 7.35 µmol^{-1} g^{-1} fw, respectively. The increase of chlorophyll a and b in leaves was 1.48 to 1.75 and 0.64 to 0.88 mg g^{-1}fw with increasing Se from1.00 to 2.0 mg/L^{-1}, respectively. These results are

agreement with the findings of **Mozafariyan et al., (2017)** who reported an increase in chlorophyll content of tomato leaves when the plants were given 7 and 10 µM of selenium. **Feng et al.,(2013**) noted that the addition of Se to the growth substrates can reduce the excess ROS generation, especially of O_2- and/or H_2O_2, in plants under stress.

Table 2. Effect of the studied treatments on chlorophyll a, chlorophyll b and proline

Treatment	Chlorophyll a, mg g^{-1}fw	Chlorophyll b, mg g^{-1}fw	Total Chlorophyll, mg g^{-1}fw	Proline, µ mol g^{-1}fw
T0	1.39	0.55	1.94	4.45
T1	1.48	0.64	2.12	6.55
T2	1.62	0.73	2.35	6.95
T3	1.75	0.88	2.63	7.35
LSD at 5%	0.65	0.22	0.76	1.25

3-3 Selenium Accumulation in Plant

Selenium contents in plants are shown in **Table 3, Fig 4and Fig5**. All organs of canola plant, as well as seeds and oil, accumulated selenium. The uptakes in shoot were 141.82 , 210.15,and 290.39 ug Se pot^{-1} for treatments of 1.00,1.50 and 2.00 mg Se L^{-1} respectively. Comparable uptake by roots were 25.07 ,43.08 and 63.13 ug Se pot^{-1} respectively.

Table 3. Effect of studied treatments on Se uptake by Canola plants

Treatment	Se uptake, µg pot^{-1}				
	Shoots	Roots	Seeds	Total	Oil
T0	4.20	2.10	1.12	7.54	0.57
T1	141.82	25.07	39.98	206.87	15
T2	210.15	43.08	62.69	315.92	26.95
T3	290.39	63.13	87.28	440.8	41.89
LSD at 5%	12.41	3.63	4.87	23.56	2.45

***selenium uptake in seeds = Se in oil + Se in The residue seed waste after Soxhlet extraction**

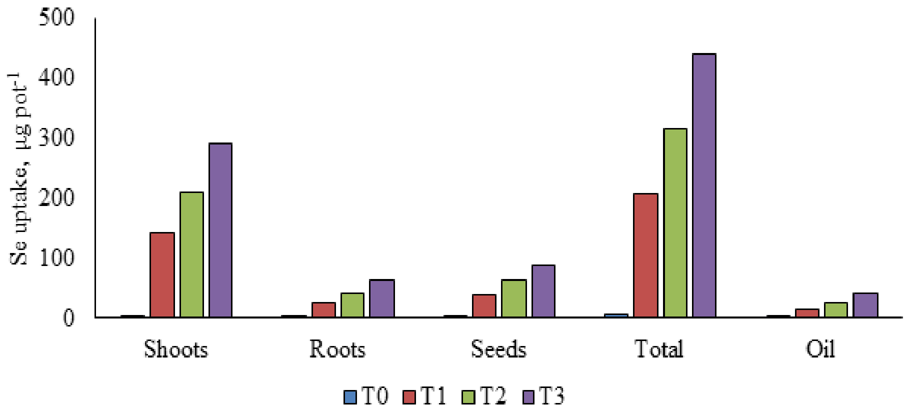

Fig. 4. Effect of the studied treatments on Se uptake by Canola plants.

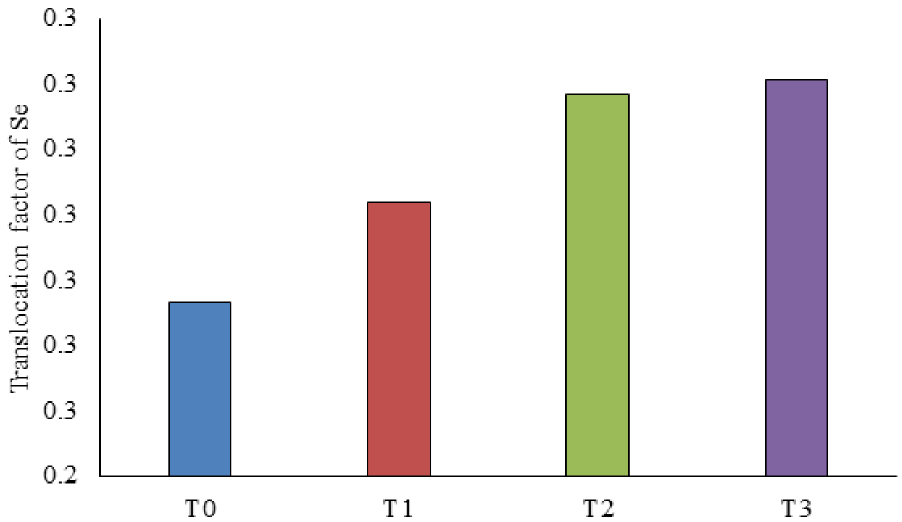

Fig. 5. Effect of the studied treatments on seed translocation factor of Se (translocation factor = seed Se content / shoots Se content)

Contents of Se in root were generally greater than in shoots. Contents in roots 9.14,13.71 and 18.29 ug g^{-1} (an average of 13.71 ug g^{-1}). Comparable contents in shoots were 8.10, 11.23 and 14.41 ug g^{-1} (average of 11.24 ug g^{-1}).

The increase in Se in oil is a direct consequence of its immersion in the Se culture. The contents in oil 15.00 ,26.95 and 41.89 ug Se g^{-1} for treatments of 1.00,1.50 and 2.00 mg Se L^{-1} respectively. The results obtained in this study agree with those obtained in other works,**(Broadley et al. 2006; Broadley et al. 2010; Ramos et al. 2010,Seppanen et al. 2010; Chilimba et al. 2012).**

Ajwa,et al .1998 ,who found that Se biofortification via crops is one of the best strategies to elevate the daily Se intake in areas where soil Se levels are low . Canola absorbs large quantities of Se. **Ebrahimi et al., 2015, 2019**, showed that the effect of Se-enriched stem or leaf residues of oilseed rape (*B. napus L*. var. Westar) increased the growth and photosynthesis of plants .Works conducted by **(Hartikainenet al., 1997; Cartes et al., 2005).** With different plants, showed that higher Se concentration with is due to greater application of Se rates.

Canola seed oil of selenium-treated plants had higher Se contents due to Se application. There may be a potential for selenium in commercially produced canola oil. Selenium is part of an enzyme called glutathione peroxidase which reduces cancer in humans **(Clark et al. 1996).** Other

researchers **(Finley et al. 1996, 1998,2005., Pappa et al .2006 and Banuelos et al., 2015)** state that Se can be given into human diets through broccoli and wheat grown on high-Se soils.The precent results show that canola oil biofortification with Se can be via done translocation under hydroponic condition .

4- CONCLUSION,

Se biofortification of canola Oil can be done when selenium is at 2 mg L^{-1} levels in the solution surrounding the roots , The weight increased with the increase in Se. The most significant result obtained from this study is that Se can get into the oil of plants grown in high-selenium Seed oil from selenium-treated plants had high Se .Se contents in oil was 15.00 ,26.95 and 41.89 ug Se g^{-1} for treatments of 1.00,1.50 and 2.00 mg Se L^{-1} respectively . Potential positive effects on getting selenium into diets of humans could potentially give canola farmers in selenium-rich regions a higher selling price

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نهج قائم على الترشيح الجذري مع السيلينيوم من أجل التقوية الحيوية لزيت الكانولا

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أجريت تجربة الأواني بالمزرعة التجريبية بكفر الحمام الزراعى. الدقة. محطة محافظة الشرقية ، مصر في موسم 2019 لتقييم استخدام الترشيح الجذري للتقوية الحيوية لزيت الكانولا (*Brassica napus* L) صنف Serw 4 .مع السيلينيوم. تمت معاملة نباتات الكانولا المزروعة في مزرعة مائية بالسيلينيوم على شكل سيلينات الصوديوم ("Na_2SeO_4") بتركيز 0 (كنترول)، 1، 1.5 و2 مجم / لتر. أوضحت النتائج وجود فروق ذات دلالة إحصائية بين المعاملات على الصفات المدروسة مع تفوق نباتات الكانولا المعاملة ب 2 مجم Se في اللتر على المعاملات الأخرى. كانت الزيادة النسبية في إجمالي الوزن الجاف للكانولا 11.36 و21.99 و31.04٪ لنباتات الكانولا المعاملة بـ 1 و 1.5 و 2 مجم Se للتر على التوالي مقارنة مع معاملة الكنترول. كانت نسبة محتوى زيت البذور 37، 40، 43 و48٪ لنباتات الكانولا المعاملة بـ 0، 1، 1.5 و2 مجم Se للتر على التوالي. كان إجمالي الكلوروفيل في الكانولا 1.94 و2.12 و2.35 و2.63 مجم / جم للوزن الطازج لنباتات الكانولا المعاملة بـ 0 و 1 و 1.5 و 2 مجم Se للتر على التوالي بينما كان البرولين 4.45 و6.55 و6.95 و7.35 ميكرمول / جم وزن طازج لنباتات الكانولا المعالمة بـ 0، 1، 1.5 و2 مجم Se للتر على التوالي. كان إجمالي امتصاص النباتات من Se هو 7.54 و 206.87 و 315.92 و 440.8 ميكروجرام لكل اصيص، بينما كان محتوى Se في الزيت 0.57 و 15 و 26.95 و 41.89 ميكروجرام للأصيص لنباتات الكانولا المعاملة بـ 0 ، 1 ، 1.5 و 2 مجم Se للتر على التوالي .